Precursor Lesions in Patients With Multiple Endocrine Neoplasia Type 1–Associated Duodenal Gastrinomas

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Background & Aims: **The identification of precursor lesions has a great impact on the understanding of tumorigenesis. Precursor lesions of endocrine tumors are known to occur in the setting of the MEN1 syndrome. The aim of this study was to test the hypothesis that MEN1-associated duodenal gastrinomas originate from diffuse preneoplastic gastrin cell changes. Precursor lesions may precede the development of duodenal gastrinomas because, in contrast to sporadic gastrinomas, these tumors are usually multiple.** ***Methods:*** **The distribution of endocrine cells in the nontumorous duodenal tissue was analyzed qualitatively and quantitatively for 25 patients operated on for a duodenal gastrinoma. MEN1 status was assessed clinically and by polymerase chain reaction–based mutational analysis.** ***Results:*** **Fourteen of 25 patients with gastrinoma had proliferative, hyperplastic lesions consisting of gastrin cells in the nontumorous duodenal mucosa, similar to the gastric enterochromaffin-like cell lesions observed in chronic atrophic gastritis. All patients with Zollinger–Ellison syndrome with proven MEN1 had such proliferative gastrin cell lesions, and all patients with Zollinger–Ellison syndrome without precursor lesions were MEN1 negative.** ***Conclusions:*** **Duodenal gastrinomas in MEN1, but not sporadic duodenal gastrinomas, are associated with proliferative gastrin cell changes within the nontumorous mucosa. It is likely that these lesions precede the development of MEN1-associated duodenal gastrinomas.**

The majority of duodenal neuroendocrine tumors are gastrinomas, which can lead to Zollinger–Ellison syndrome (ZES).[1–3] Duodenal gastrinomas may occur sporadically or in the setting of the MEN1 syndrome. Although these tumors are usually very small (≥2 mm) and grow slowly, metastasis to regional lymph nodes is common at the time of diagnosis.[4–10]

For diagnosis and therapy and for the understanding of the tumorigenesis of malignant tumors, it is particularly important to identify precursor lesions that represent a sequence of cellular changes from hyperplasia to neoplasia. There are several endocrine tumors that develop on the basis of hyperplastic changes (ie, medullary thyroid carcinoma or pheochromocytoma). Furthermore, it is likely that the adenomatous changes of the parathyroid in patients with the MEN1 syndrome originate from hyperplastic changes.[11–13] All such conditions, except for the enterochromaffin-like (ECL) cell hyperplasia in chronic atrophic gastritis, are associated with an inherited endocrine disorder.[14–16]

In 1990, it was noticed that many duodenal gastrinomas arising in the setting of MEN1 are multiple, in contrast to sporadic gastrinomas.[4] Our working hypothesis is that these multifocal gastrinomas originate from diffuse proliferative changes of the duodenal gastrin cells. The aim of this study was therefore to (1) screen the duodenum of patients with gastrinomas for preneoplastic changes in gastrin cells, (2) quantify their extent and proliferative capacity, and (3) determine whether they are associated with the MEN1 syndrome.

Materials and Methods

Patients

The study included 25 patients treated for duodenal gastrinomas whose specimens were collected between 1980 and 2002 in the consultation files of the departments of pathology of the University of Kiel (Germany) and the University of Zurich (Switzerland). Three patients underwent endoscopic polypectomy, 14 patients were treated by local excision, 3 patients underwent partial duodenectomy, and 5 patients had a Whipple resection. In addition to the duodenal gastrinomas, the presence of other endocrine tumors known to

Abbreviations used in this paper: CgA, chromogranin A; DGGE, denaturing gradient gel electrophoresis; ECL, enterochromaffin-like; PCR, polymerase chain reaction; TBS, Tris-buffered saline; ZES, Zollinger–Ellison syndrome.

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Table 1. List of Primary Antibodies

Antigen	Code	Source/reference	Dilution	Species
Gastrin	G 17	Paesel, Frankfurt, Germany	1:3000/1:200[a]	Rabbit, polyclonal
Cholecystokinin	CCK-8 YP030	Yanaihara, Shizuoka, Japan	1:40,000	Rabbit, polyclonal
Motilin	Y120	Yanaihara, Shizuoka, Japan	1:40,000	Rabbit, polyclonal
Secretin	Y030	Yanaihara, Shizuoka, Japan	1:40,000	Rabbit, polyclonal
Xenin	2815/3	Anlauf et al[35]	1:6000	Rabbit, polyclonal
Somatostatin	A 0566	Dako, Hamburg, Germany	1:200	Rabbit, polyclonal
Gastric inhibitory peptide	GIP Y101	Yanaihara, Shizuoka, Japan	1:50,000	Rabbit, polyclonal
Serotonin	5HT H209	Dako, Hamburg, Germany	1:20	Mouse, monoclonal
Serotonin	5HT 43H37R	INC/IBL, Hamburg, Germany	1:60,000	Rabbit, molyclonal
Vesicular monoamine transporter 1	1/10	Erickson et al[36]	1:2000	Rabbit, molyclonal
α–human chorionic gonadotropin	823	Biogenex, San Ramon, CA	1:100	Mouse, monoclonal
CgA	E 001	Linaris, Wertheim, Germany	1:2/1:1[a]	Mouse, monoclonal
Synaptophysin	A0010	Dako, Hamburg, Germany	1:50	Rabbit, polyclonal
Ki-67	Ki-67	Kreipe et al[37]	1:1/1:30[a]	Mouse, monoclonal

[a]Immunofluorescence.

occur in patients with the MEN1 syndrome was recorded. An MEN1 status was assessed if at least 2 main MEN1-related endocrine tumors (parathyroid adenoma, enteropancreatic endocrine tumors, pituitary tumors, adrenocortical tumors, foregut carcinoids) were present. Patients with multifocal or recurrent endocrine tumors were defined as clinically suspicious for MEN1.[17]

Materials

From each patient, representative paraffin-embedded tissue blocks containing tumor and extratumoral duodenal mucosa fixed in either 4% formaldehyde or Bouin's solution were examined. All gastrin cell tumors >300 μm and associated with a ZES were classified as gastrinomas. Tumors >2 mm were referred to as macrotumors and tumors from 300 μm to 2 mm as microtumors. The occurrence of 2 or more duodenal gastrinomas was defined as multifocality. Samples from different regions of the duodenum of 3 patients who had a Whipple resection for pancreatic ductal adenocarcinoma (3 men; mean age, 53 years; range, 39–69 years) were used as control tissue.

Immunohistochemistry

The duodenal endocrine cell populations were examined by staining with the antibodies listed in Table 1. To assess proliferation in gastrinomas and in extratumorous gastrin cells, the expression of the nuclear proliferation antigen Ki-67 was determined (Table 1).

Deparaffinized sections (3–4 μm thick) were rehydrated and subjected to heat-induced epitope retrieval procedures as previously described.[18,19] Before application of the primary antibody, blocking with nonimmune serum was performed for 20 minutes. After incubation for 45 minutes, the reaction was detected with species-specific biotinylated secondary antibodies (Dianova, Hamburg, Germany) for 45 minutes, washed several times in phosphate-buffered saline, and incubated for 30 minutes with the ABC reagents (Vectastatin Elite ABC kit; Boehringer, Ingelheim, Germany). Immunoreactions were visualized with 3,3′-diaminobenzidine tetrahydrochloride (Sigma, Deisenhofen, Germany), which resulted in brown staining. The number of gastrin- and chromogranin A (CgA)-positive cells in neuroendocrine tumors was estimated semiquantitatively as an approximate percentage and scored on a scale from 1 (<20%) to 5 (80%–<100%). To examine nuclear expression of Ki-67 in gastrinomas, 20 high-power fields (40× lens) were analyzed. Immunostained sections were analyzed and photographed with an Axioskop 50 microscope (Zeiss, Oberkochen, Germany).

Assessment of Nontumorous Gastrin Cell Lesions

The gastrin cell lesions were classified in a double-blind fashion by 2 observers in analogy to the system proposed for the ECL cells of the stomach.[20] In most samples, at least 10 serial sections at a distance of more than 0.5 cm from the tumor were assessed. The frequency of gastrin cells in the duodenal crypts and Brunner's glands was scored using a scale from 0 to 6 (0, absent; 1, <10%; 2, 10%–<20%; 3, 20%–<40%; 4, 40%–<60%; 5, 60%–<80%; 6, 80%–100%).

Morphometric Analysis

Gastrin cell density was analyzed in 3 patients with gastrinoma with associated nontumorous gastrin cell lesions versus 3 patients with gastrinoma without any evidence of nontumorous gastrin cell lesions and 3 control patients. For each patient, 3 tissue samples obtained from the first portion of the duodenum at least 0.5 cm from the excised tumor were examined. Cell density was determined by the hot spot method as previously described.[21,22] In crypts and Brunner's glands, gastrin-immunoreactive cells showing a clearly visible nucleus were counted (40× lens) and expressed as number of mucosal cells per millimeter of the muscularis mucosae and number of cells per square millimeter of Brunner's glands, as described in earlier studies.[23,24] The length of the muscularis mucosae and the area of Brunner's glands were determined with an occulometer (Netzmicron; Carl Zeiss, Jena, Germany). The minimum length of duodenal mucosa examined per case was 2 cm,